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(54) Title: PROTECTIVE ROLE OF POLYAMINES IN MODIFICATIONS OF BASEMENT MEMBRANE MACROMOLECULES

(57) Abstract

Polyamines including putrescine are found to interfere with nonenzymatic glucosylation, interfere with formation of advanced glucosylation end-products by minimizing crosslink formation and act as a reducing agent to minimize oxidative damage to proteins. These polyamines are useful in treating diabetics to minimize damage caused by the high glucose concentrations and to reduce cross-linking-mediated or oxidation-mediated tissue aging.

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PROTECTIVE ROLE OF POLYAMINES IN MODIFICATIONS OF BASEMENT MEMBRANE MACROMOLECULES

Background of the Invention

1. Field of the Invention

This invention relates to polyamine compositions useful in preventing diabetic complications caused by nonenzymatic glucosylation, cross-linking and oxidative damage and tissue changes like cross-linking and oxidative alterations that are contributors to the aging process.

2. Description of the Related Art

Basement membranes are multifunctional specializations of the extracellular matrix. They are found either at the basal surface of various cell types that exhibit polarity (epithelial, mesothelial, endothelial cells) or surround various other cell types (muscle cells, adipose cells, Schwann cells). Various biologically significant roles have been ascribed to basement membranes: compartmentalization of tissues, contribution in cell anchorage and in the maintenance of cell polarity, control of cell migration, involvement in invasion of normal and malignant cells, and as a permeability barrier to macromolecules [Vracko, R (1974) Am. J. Pathol. 77:314-388; Timpl, R. and Dziadek, M. (1986) Inl. Rev. Exp. Pathol. 29: 1- 112]. This last function is of utmost importance in the kidney glomerulus, where the glomerular basement membrane is the only barrier to extravasation of circulating macromolecules. In diabetes, the impaired function of the glomerular basement membrane leads to proteinuria.

In order to better understand the structure and function of basement membranes and the molecular mechanism(s) underlying the diabetic alterations, it is essential to know their macromolecular components and how they interact to form the final structure. Difficulties in extracting these macromolecules in high purity and high yield have led to the identification of the Engelbreth-Holm-Swarm (EHS) tumor. This is a murine non-invasive tumor that secretes in large amounts a matrix consisting almost exclusively of basement membrane macromolecules [Orkin, R.W., et al. (1977) J. Exp. Med. 145:204-220]. Results from this system, later confirmed with other bona fide basement membranes indicate that three types of macromolecules are mainly

present as structural components exclusively found in basement membranes: (a) collagenous glycoproteins, of which type IV collagen is by far the most abundant [Timpl. R. et al. (1979) Eur. J. Biochem. 84:43-52]; (b) non collagenous glycoproteins, of which laminin is the most prominent and well studied [Timpl, R. et al (1979) J. Biol. Chem. 254:9933-9937]; and (c) proteoglycans, mainly of the heparan sulfate type [Hassel, J.R., et al. (1985) J. Biol. Chem. 260:8098-8105].

The isolation of these macromolecules in large amounts and in pure form allowed many functional studies that analyzed in detail their interactions in vitro [Charonis, A.S., and Tsilibary, E.C. (1990) "Assembly of basement membrane proteins" pp. 85-117, in *Organization and Assembly of Plant and Animal Extracellular Matrix.* Mecham, B., and Adair, S., editors, Academic Press]. It is well established that both laminin [Yurchenco, P.D., et al. (1985) J. Biol. Chem. 262:7636-7644] and type IV collagen [Yurchenco, P.D., and Furthmayr, H. (1984) Biochemistry 23:1839-1850] have the ability to self-associate and to interact with each other [Charonis, A.S., et al. (1985) J. Cell Biol. 100:1848-1853] and with heparan sulfate proteoglycan [Laurie, G.W., et al. (1986) J. Mol. Biol. 189:205-216].

In diabetes, hyperglycemia affects many metabolic pathways and each of these changes may contribute to the development of diabetic complications. The basis of many diabetic complications is microangiopathy, and microangiopathy is characterized by morphological and physiological changes in the basement membrane underlying endothelial cells of the microvasculature.

We have focused on three related macromolecular alterations observed under diabetic conditions, which are generated by high glucose concentrations: (a) nonenzymatic glucosylation, (b) formation of advanced glucosylation end-products (crosslinking) and (c) oxidative damage/degradation.

(a) nonenzymatic glucosylation

Glucose has the ability to attach chemically to proteins without the participation of enzymes. This reaction occurs normally but at very slow rates when compared to hyperglycemic conditions, and is known as Maillard reaction or nonenzymatic glucosylation [Day, J.F., et al. (1979) J. Biol. Chem. 254:595-597; Brownlee, M., et al. (1984) Ann. Intern. Med. 101:527-537]. The site of nonenzymatic glucosylation is either at the N-terminal amino acid or at the epsilon

amino group of lysine residues, which is by far the more common site. Initially a Schiff base product is formed and subsequently, at a slower rate, an Amadori product is created. Both reactions are reversible, however, the Amadori product is much more stable than the Schiff product.

Nonenzymatic glucosylation of proteins occurs under normal conditions and is thought to play a role in the process of aging; this process is accelerated as a consequence of the hyperglycemic status in diabetes. It has been reported for many proteins such as hemoglobin [Bunn, H.F., et al. (1979) J. Biol. Chem. 254:3892-3898; Shapiro, R., et al. (1980) J. Biol. Chem. 255:3120-3127], albumin [Day, J.F., et al. (1979) J. Biol. Chem. 254:595-597; Day, J.F., et al. (1980) J. Biol. Chem. 255:9394-9400], low density lipoproteins [Gonen, B., et al. (1981) Diabetes 30:875-878], lens crystallins [Stevens, V.I., et al. (1978) Proc. Natl. Acad. Sci. USA 75:2918-2922], fibronectin [Tarsio, J.F., et al. (1985) Diabetes 34:477-484], basement membrane collagen [Cohen, M.P., and Wu, V.-Y. (1980) Biochem. Biophys. Res. Comm. 100:1549-1554]. It has been shown that nonenzymatically glucosylated proteins may exhibit altered physiochemical properties. For example, hemoglobin has lower affinity for oxygen [McDonald, M.J., et al. (1979) J. Biol. Chem. 254:702-707], albumin has decreased ability for bilirubin [Shaklai, N., et al. (1984) J. Biol. Chem. 259:3812-3817], lens crystallins may aggregate and cause opalescence [Monnier, V.M., et al. (1979) J. Exp. Med. 150: 1098- 1107]. We have observed that nonenzymatic glucosylation of domain NC 1 of type IV collagen leads to defective association between this domain and binding sites along the length of its triple helical portion and therefore it interferes with its ability to polymerize [Tsilibary, E.C., et al. (1988) J. Biol. Chem. 263:4302-4308]. In conclusion, nonenzymatic glucosylation may lead to structural changes that may affect important functions. In the case of the basement membrane macromolecules, it may affect their assembly process and therefore their supramolecular organization.

(b) Formation of advanced glucosylation end-products (cross-linking)

It has been established that nonenzymatic glucosylation occuring either as part of the aging process or in diabetics proceeds further than the Amadori products and crosslinks develop, either between two Amadori products or between an Amadori product and an unmodified lysine residue [Brownlee, M. et al. (1984) Ann. Intern.

Med. 101:527-537]. The first such advanced glucosylation end-product to be characterized has the structure 2-furoyl-4(5)-(2-furanyl)-1H-imidazole, known also as FFI [Pongor, S. et al. (1984) Proc. Natl. Acad. Sci. (USA) 81:2684-2688]. Eventually, other crosslinked products have been or are being characterized [Baynes, J.W. et al. (1990) in *Glycated Proteins in Diabetes Mellitus;* Sell. D.R., and Monnier, V.M. (1989) J. Biol. Chem. 264:21597-21602]. Formation of crosslinks, both intra- and inter-molecular in nature, has been postulated as one major parameter in the process of aging. This process is considered very complicated and the development of crosslinks can be due to nonenzymatic as well as enzymatic mechanisms, the nature of which is largely unknown.

(c) Oxidative damage/degradation

Glucose and monosaccharides in general can reduce molecular oxygen and yield H_2O_2 and free radical intermediates [Thornalley, P. et al. (1984) Biochim. Biophys. Acta 797:276-287]. These compounds can lead to fragmentation of proteins [Hunt, J.V., et al. (1988) Biochem. J. 250:87-93]. This process is catalyzed by Cu^{++} and can be inhibited by metal chelating agents. Although there is no direct evidence of this process in diabetes, there are observations supporting the idea that hyperglycemia induced oxidative stress is a factor in the pathogenesis of diabetic complications: antioxidants such as ascorbic acid, vitamin E or glutathione are decreased and plasma Cu^{++} is increased in diabetes.

Efforts to introduce treatments that will interfere with any of the three hyperglycemia induced macromolecular alterations discussed above are very intense. Recently, it has been suggested the aminoguanidine, a nucleophilic hydrazine compound, can prevent diabetically induced protein crosslinking, both *in vitro* and *in vivo* [Brownlee, M., et al. (1986) Science 232: 1629- 1632] *See* also U.S. Patents 4,983,604; 4,908,446 and 4,758,583. However, conflicting reports exist as to the effect of aminoguanidine on glucose-incorporation to proteins. The original report suggested that aminoguanidine does not interfere with the first step of nonenzymatic glucosylation, the formation of Amadori products. In contrast, another **group** has observed that aminoguanidine can have a major effect in lowering glucose concentration, by reacting directly with glucose [Khatami, M., et al. (1988) Life Sciences 43:1725-1731].

These differences may be due to the various concentrations used. Whatever the mechanism or mechanisms of action of aminoguanidine could be, a lot of caution is required for its future use as a potential pharmaceutical agent because it is a chemical foreign to the human body.

The art described in this section is not intended to constitute an admission that any patent, publication or other information referred to herein is "prior art" with respect to this invention, unless specifically designated as such. In addition, this section should not be construed to mean that a search has been made or that no other pertinent information as defined in 37 C.F.R. § 1.56(a) exists.

Summary of the Invention

We have tested the effect of polyamines, in the glucose-induced changes of macromolecules, as described above. We have selected putrescine as a representation of the group of naturally occurring polyamines, reasoning that these compounds will have minimal, if any, toxic effects if administered systematically, even at very high concentrations (see below).

We have found that:

1) Polyamines, including putrescine, interfere with the nonenzymatic glucosylation by reducing to some extent the amount of glucose incorporated to proteins.

2) Polyamines, including putrescine, interfere with formation of advanced glucosylation end-products by minimizing the extent of crosslink formation.

3) Polyamines, including putrescine, may act to minimize the oxidative damage to proteins, by acting as a reducing agent.

Therefore, the polyamines of the invention are useful in preventing glucose-induced changes in macromolecules. This means that the compositions may be used beneficially to treat a variety of diseases or aging in which damage is associated with glucose-induced macromolecule changes. In fact, the compositions are useful whenever protein damage is to be minimized, and therefore includes applications to retard food spoilage.

Several compounds of the invention are well known and may be readily

obtained or prepared from available base chemicals. The other compounds of the invention may be readily prepared from base materials.

Brief Description of the Drawing

A detailed description of the invention is hereafter described with specific reference being made to the drawing in which:

FIG. 1 shows crosslink formation using gel electrophoresis with separate gel lanes for each sample.

Description of the Preferred Embodiments

Polyamines are low molecular weight aliphatic nitrogenous bases. The three common polyamines are: putrescine with the formula $NH_2(CH_2)_4NH_2$; spermidine with the formula $NH_2(CH_2)_3NH(CH_2)_4NH_2$; and spermine with the formula $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$. Although other less common polyamines exist, mainly in plants and microorganisms, the above described polyamines are present in every living organism and often their intracellular concentration reaches millimolar amounts [Morgan, D.M.L. (1987) Essays in Biochem. 23:82-115]. Some of the less common polyamines include the diamines such as 1,3-diaminopropane and cadaverine; triamines such as norspermidine, aminopropylcadaverine and homospermidine; tetra-amines such as norspermine, thermospermine and canavalmine; and penta-amines including caldopentamine and homocaldopentamine.

We have focussed on the effects of putrescine, the simplest member of this group. The pK of the amino group of putrescine is 8.71; the pKs of the amino and imino groups of spermine and spermidine exhibit on the average much higher values, therefore they should be considered stronger bases. If these values are crucial for the phenomena studied below, it is believed that the action of spermine and spermidine will be even more dramatic than the one of putrescine.

An impressive feature of these compounds may be the fact that they could be administered at very high doses, without having any deleterious side effect. In a recent report [Manni, A. et al (1986) Cancer Res.46:4938-4941] putrescine was used in rats at doses as high as 500 mg per kg of body weight per day. This dose, assuming equal tolerance in humans is equivalent to 35 grams per day for an average individual.

It is claimed that compounds with the chemical formula:

$$NH_2[(CH_2)_a(NH)_b]_x(CH_2)_4[(NH)_c(CH_2)_d]_yNH_2$$

where a, b, c, d, x, and y can take any value ≥ 0, and most preferably where a, b, c, d, x, and y can take any value ≤ 4, ≥ 0 may interfere with and produce a beneficial effect in phenomena such as nonenzymatic glucosylation, formation of advanced glucosylation endproducts, and oxidation of biological macromolecules. The compositions may also be useful with substitutions in place of one or more hydrogen atoms. It has been found, however, that introduction of a carboxyl group will very markedly lower the usefulness of the compound.

The invention will be described in detail by using the following detailed examples. In all these examples we have used as a model system one basement membrane glycoprotein, laminin. For the experiments described below, the source of laminin was the matrix of the Engelbreth-Holm-Swarm tumor, grown subcutaneous in mice [Orkin, R.W., et al (1977) J. Exp. Med. 145:204-220]. Laminin was extracted and purified following well-established techniques [Charonis, A.S., et al. (1985) J. Cell Biol. 100:1848-1853].

Example 1 - Glucose incorporation in the presence of putrescine

Laminin, at a concentration of 300 μg/ml was incubated in the presence of 0.5M radioactive glucose alone or in the presence of various concentrations of putrescine (5 mM, 50 mM, 500 mM) and aminoguanidine (500 mM). The samples were kept at 37 °C for 21 days in the dark, with occasional shaking. At the end of the incubation period aliquots from each sample were dialyzed extensively in buffers containing denaturing agents to remove radioactivity loosely associated with laminin and finally every aliquot was counted to determine its radioactivity and its protein concentration. The results from this experiment are shown in Table 1.

Table 1

Description of sample	cpm glucose per microgram of protein
Laminin + glucose	447
Laminin + glucose + putrescine 5 mM	328
Laminin + glucose + putrescine 50 mM	309
Laminin + glucose + putrescine 500 mM	263
Laminin + glucose + aminoguanidine 500 mM	290

These results suggest that putrescine has the ability to prevent to some extent the nonenzymatic glucosylation of proteins, in a concentration dependent manner. The results also demonstrate that aminoguanidine has the same effect, although it may be slightly less potent than putrescine in that effect.

Example 2 - Crosslink formation in the presence of putrescine

Laminin, at a concentration of 500 μg/ml was incubated in the presence or absence of 0.5M glucose, and in the absence or presence of increasing putrescine concentrations (5 mM, 50 mM, 500 mM). Samples were incubated for 21 days at 37 °C in the dark with occasional shaking. At the end of the incubation period aliquots from each sample were dialyzed in phosphate buffered saline to remove all the small molecular weight components, their protein concentration was determined, and equal amounts of protein were analyzed by gel electrophoresis. In this experiment, highly crosslinked material is not able to enter the running gel and is observed as a band at the bottom of the stacking gel. The results of this experiment, after staining the gel with Coomassie Blue, are shown in Figure 1. The arrows indicate the position of high molecular weight crosslinked material. Lane a is plain laminin. Lane b is laminin in the presence of glucose. Lane c is laminin in the absence of glucose and in the presence of 500 mM putrescine. Lane d is laminin in the presence of glucose and 500 mM putrescine. Lane e is laminin in the presence of glucose and 50 mM putrescine. Lane f is laminin in the presence of glucose and 5 mM putrescine. The results from Figure 1 show that putrescine could effectively inhibit the formation of crosslinks in a concentration-dependent fashion. In order to obtain a more quantitative assessment of

the effect of putrescine on crosslink formation, the lanes of the gel shown in Figure 1 were analyzed densitometrically. The crosslinks in the control lane, lane a, were assigned a value of 0%. The crosslinks in lane b, in the absence of putrescine, were assigned a value of 100%. The results of the densitometric analysis are shown in Table 2.

Table 2

Sample	Percent of maximal crosslinking
Laminin	0.0
Laminin + glucose	100.0
Laminin + putrescine (500 mM)	0.0
Laminin + glucose + putrescine (500 mM)	0.0
Laminin + glucose + putrescine (50 mM)	13.76
Laminin + glucose + putrescine (5 mM)	49.36

These data demonstrate that putrescine is a very potent inhibitor of formation of crosslinks.

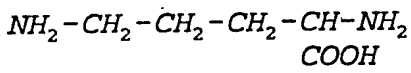
Example 3 - Effect of putrescine on protein degradation

The data of the experiment described in Example 2 were used to assess whether putrescine is able to interfere with and protect from degradation. To that purpose, the intactness of laminin after incubation in the absence (lane a of Figure 1) or the presence of putrescine (lane c of Figure 1) was measured densitometrically. The results are presented in Figure 2. The top panel is lane a and the bottom panel is lane c. Both Figure 1 and Figure 2 show that the presence of putrescine allowed for a better preservation of laminin A and B chains.

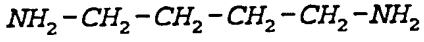
This finding corroborates a recent report in the literature [Mizui, T., et al. (1987) Japan. J. Pharmacol. 44:43-50] showing that all three polyamines can protect from oxidative damage due to their antiperoxidative properties.

Example 4 - Comparison of putrescine and lysine

Because lysine



has a structure with similarities to putrescine



we decided to examine its effect on crosslink formation. Laminin at a concentration of 400 μg/ml was incubated in the presence or absence of glucose (500mM), putrescine (50mM), or lysine (50mM), as described in Example 2. At the end of the experiment, samples were run on gel electrophoresis under reducing conditions and the highly crosslinked material that does not enter the running gel was quantitated by densitometric analysis. In order to accurately quantitate the extent of crosslinking, in each case the density of every sample in which glucose was present was reduced by the density of the same sample in which glucose was omitted. The density of laminin plus glucose was assigned the value of 100%. The results are shown in Table 3.

Table 3

Sample	Percent of Crosslinking
Laminin + glucose	100.00 %
Laminin + glucose + putrescine	20.83 %
Laminin + glucose + lysine	70.83 %

The results of Table 3 demonstrate that putrescine is by far a more effective agent in preventing crosslinking, compared to lysine. The structural similarity of these two compounds suggest that the absence of the carboxyl group may be very crucial in the anti-crosslinking action. It is important to note in this regard, that because of this carboxyl group the pI of lysine (7.22) is far lower that the pI of putrescine (8.71) and even more dramatically lower compared to the pI of other naturally occurring polyamines (spermidine, spermine) whose structure is included in the general formula claimed.

Usages of the Compositions

Diabetics or others wishing to decrease nonenzymatic glucosylation, reduce crosslink formation of advanced glucosylation end-products or reduce oxidative damage to proteins may be treated with compounds of the invention. The treatment involves introduction of a polyamine of the invention into the individual at an effective amount. This may be given one or more times daily, and may involve dosages that would provide an effective amount of polyamine in the blood of up to 500 mM.

This therapy may be used to decrease nonenzymatic glucosylation, reduce crosslink formation of advanced glucosylation end-products or reduce oxidative damage to proteins associated with aging, diabetes or even food spoilage. In clinical applications the compounds may be formulated for topical, oral or parenteral administration with the usual carriers or diluents.

Pharmaceutical application of the compounds may be made with carriers selected from known materials. The compounds may be converted to hydrochloride salts from bicarbonate salts to improve solubility for intraperitoneal injection. Other pharmaceutically acceptable acid addition salts of the compositions of the invention may be formulated. These salts include derivation from organic and inorganic acids such as sulfuric, phosphoric, p-toluenesulfonic, hydrochloric, hydrobromic, sulfamic, citric, lactic, maleic, benzoic, ascorbic and related acids.

The composition may be formulated as a liquid for intravenous, intraperitoneal or oral administration. Topical usages as an anti-aging formulation may be useful, in which case the compositions may be compounded as ointments or cremes.

The expected dosage may be as high as 10 g/day for humans, and more typically up to 0.1 g/kg of body weight. Even higher dosages may be beneficial and are not expected to cause any deleterious side effects. The inventors best estimate on the upper useful range of application is given to satisfy the disclosure requirements and does not necessarily represent absolute limits.

While this invention may be embodied in many different forms, there are shown in the drawings and described in detail herein specific preferred embodiments of the invention. The present disclosure is an exemplification of the principles of the invention and is not intended to limit the invention to the particular embodiments illustrated.

This completes the description of the preferred and alternate

embodiments of the invention. Those skilled in the art may recognize other equivalents to the specific embodiment described herein which equivalents are intended to be encompassed by the claims attached hereto.

WHAT IS CLAIMED IS:

1. Compounds of the formula:

$$NH_2[(CH_2)_a(NH)_b]_x(CH_2)_4[(NH)_c(CH_2)_d]_yNH_2$$

where a, b, c, d, x, and y are ≥ 0 and ≤ 4 and pharmaceutically acceptable acid addition salts in a pharmaceutically effective amount for reducing nonenzymatic glucosylation, formation of advanced glucosylation cross-linking and oxidative damage of a target protein within said animal.

2. The compound of Claim 1 wherein a, b, d, x and y are 0.

3. Putrescine in a pharmaceutically effective amount for reducing nonenzymatic glucosylation, formation of advanced glucosylation cross-linking and oxidative damage of a target protein within said animal:

$$NH_2-CH_2-CH_2-CH_2-CH_2-NH_2$$

and its pharmaceutically acceptable acid addition salts together with a pharmaceutically acceptable carrier.

4. Polyamines useful in lessening diabetic modifications of basement membrane macromolecules including putrescine, spermine and spermidine and their pharmaceutically acceptable acid addition salts together with a pharmaceutically acceptable carrier.

5. Polyamines useful in lessening diabetic modifications of basement membrane macromolecules including

$$NH_2[(CH_2)_a(NH)_b]_x(CH_2)_4[(NH)_c(CH_2)_d]_yNH_2$$

where a, b, c, d, x, and y are greater than or equal to 0 and less than or equal to 4, including substituent groups wherein the pI of the substituted polyamines is greater than about 8.00; pK greater than about 8.5 and their pharmaceutically acceptable acid

addition salts together with a pharmaceutically acceptable carrier.

6. A method for treating diabetics comprising administering a protective dose of:

$$NH_2 [(CH_2)_a (NH)_b]_x (CH_2)_4 [(NH)_c (CH_2)_d]_y NH_2$$

or its pharmaceutically acceptable acid addition salts together with a pharmaceutically acceptable carrier.

7. A method for treating an animal to inhibit nonenzymatic glucosylation, formation of advanced glucosylation cross-linking and oxidative damage of a target protein within said animal, the method comprising administration of an effective amount of a pharmaceutical composition comprising a compound of the formula:

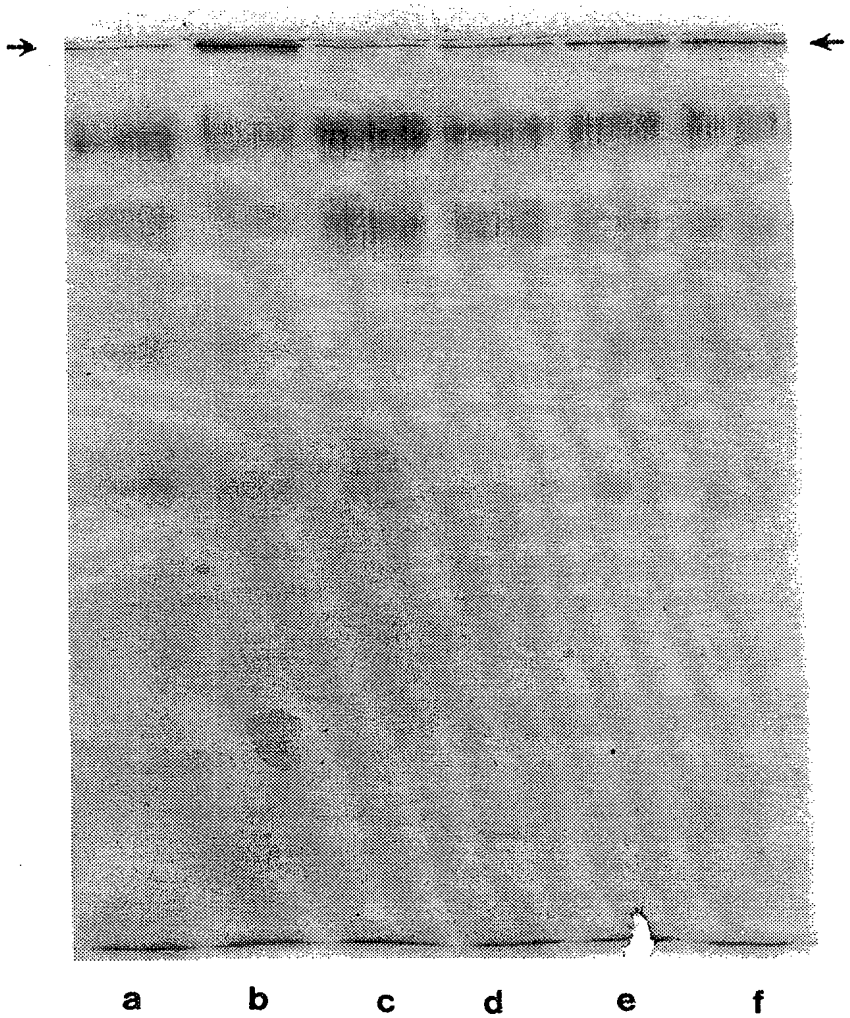
$$NH_2 [(CH_2)_a (NH)_b]_x (CH_2)_4 [(NH)_c (CH_2)_d]_y NH_2$$

where a, b, c, d, x, and y are greater than or equal to 0 and less than or equal to 4 and its pharmaceutically acceptable acid addition salts.

8. The method of Claim 7 wherein said compound is a polyamine selected from the group consisting of putrescine, spermine and spermidine.

9. The method of Claim 7 wherein said pharmaceutical composition is administered orally at a dosage of up to 0.1 g/kg of body weight of said animal.

Fig. 1



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11769

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C07C 211/09, 211/13, 211/14; A61K 31/13
US CL :564/511, 512; 514/673, 674
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 564/511, 512; 514/673, 674

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,077,313 (LUBEC) 31 DECEMBER 1991, see entire document.	1, 4-9
X	JP, A, 52-099,224 (DAINIPPON PHARM KK) 19 Ausust 1977, see entire document.	1-5
X Y	Chemical Abstracts, Volume 80, No. 21, issued 27 May 1974, Hayashi, "Pharmacological and Physiological Actions of Polyamines," see page 2, column 2, abstract no. 115852y, Taisha, 9(11), 1026-32 (1972).	1 4,5

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search
19 January 1994

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Int ational application No.
PCT/US93/11769

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAISHA, Volume 9, No. 11, issued 1972, Hayashi, "Pharmacological and Physiological Actions of Polyamines," pages 1026-32, see entire document.	1-5

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US93/11769

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAS ONLINE, BIOSIS, MEDLINE
search terms: spermine, putrescine, spermidine, polyamines, diamines, glucosylate, glycosylate, diabetes, radical scavenger, aging